0273-2289/1559–0291 (Online)/07/136–140/897–908/$30.00

Semicontinuous Production of Lactic Acid From Cheese Whey Using Integrated Membrane Reactor

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Abstract

Semicontinuous production of lactic acid from cheese whey using free cells of *Bifidobacterium longum* with and without nanofiltration was studied. For the semicontinuous fermentation without membrane separation, the lactic acid productivity of the second and third runs is much lower than the first run. The semicontinuous fermentation with nanoseparation was run semicontinuously for 72 h with lactic acid to be harvested every 24 h using a nanofiltration membrane unit. The cells and unutilized lactose were kept in the reactor and mixed with newly added cheese whey in the subsequent runs. Slight increase in the lactic acid productivity was observed in the second and third runs during the semicontinuous fermentation with nanofiltration. It can be concluded that nanoseparation could improve the lactic acid productivity of the semicontinuous fermentation process.

Index Entries: Cheese whey; fermentation; lactic acid; lactose; membrane; nanofiltration; semicontinuous.

Introduction

Lactic acid is a natural organic acid and has many applications in the pharmaceutical, food, and chemical industries. It is used as acidulant and preservative, and recently its potential as substrate for the production of biodegradable plastic has been actively pursued *(1,2)*. Approximately half of the world's supply of lactate is produced by fermentation process. Although batch fermentation processes are currently used, a number of more advanced techniques have been investigated in order to improve the process efficiency *(3)*. Semicontinuous and continuous lactic acid production systems have been recently studied to improve the fermentation performance *(4–6)*. Cheese whey is an important byproduct from the cheese manufacturing industry. Typically, 100.0 g of milk yield, 10.0 g of cheese, and 90.0 g of

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liquid whey *(7)*. Cheese whey contains about 4.5–5% lactose, 0.6–0.8% soluble proteins, 0.4–0.5% (w/v) lipids, and varying concentrations of mineral salts *(8)*. Therefore, there is an interest to utilize lactose from cheese whey in the production of value-added products such as lactic acid.

Lactic acid has been produced by fermenta tion of sugar-containing substrates such as cheese whey using *Lactobacillus helveticus (2,9)* and *L. casei (4,5)* in most of the previous studies. *Bifidobacterium longum* is a bacterium that can convert lactose into lactic acid and also produce an antibacterial compound, which can boost the immune system in its host. In recent years, there has been an increasing interest in the incorporation of the intestinal bacterial species, *B. longum*, into fermented milk products. Studies have demonstrated that *B. longum* in fermented milks have a variety of beneficial health effects in human and animal intestinal tract *(10,11)*. Most previous studies on the fermentation process using *B. longum* have focused on the production of *B. longum* cell *(12,13)* for application in the food and pharmaceutical industry. The research on lactic acid production using *B. longum* is scarce.

Nanofiltration is a pressure-driven membrane process with a MWCO situated between reverse osmosis and ultrafiltration. The nanofiltration membrane has already been used in the demineralization of salted, acid, and sweet cheese whey *(14)*. The process could separate monovalent salts and organics in the molecular weight range 200–1000 Da *(15)*. Nanofiltration membrane with MWCO around 400 Da was demonstrated to retain about 97% of lactose and 12–35% of lactate at pH 3.3 in a nanofiltration membrane-reactor *(1)*. Nanofiltration of cheese whey has been evaluated based on the permeate flux to improve the demineralization rate by Alkhatim et al. *(16)*. The objectives of this study were: (a) to evaluate the performance of nanofiltration filtration membrane on the lactic acid purification, and (b) to evaluate the performance of semicontinuous production of lactic acid from cheese whey with and without nanofiltration.

Materials and Methods

Cheese Whey Media

Cheese whey media was prepared by dissolving 50.0 g of deproteinized cheese whey powder (Davisco Foods International Inc., Eden Prairie, MN) into a liter of deionized water and stirring for 5 min at ambient temperature to obtain 5% cheese whey concentration. The composition of the deproteinized cheese whey powder was as follows: crude protein (total nitrogen × 6.38) 6.8%, crude fat 0.8%, lactose 78.6%, ash 9.4%, and moisture 4.4%. The solutions were autoclaved at 103°C for 10 min before being used at the fermentation experiments.

Microorganism and Culture Media

B. longum was obtained from the National Collection of Food Bacteria (NCFB 2259). Stock culture of this strain was maintained in 50% glycerol

and Man Rogosa Sharpe (MRS) broth media at –80°C. Active cultures were propagated in 10 mL MRS broth at a temperature of 37°C for 24 h under anaerobic conditions. This was used as a preculture to initiate cell production of higher volume with a 1% inoculation into 100 mL fresh MRS broth, and incubated at 37°C for 24 h.

Nanofiltration

The nanofiltration system consisted of a recirculation pump, nanofiltration unit (SEPA CF II, Osmonics, Minneapolis, MN), and an online permeate weighting unit. The cheese whey broth was circulated from the fermentor to the nanofiltration membrane unit at a constant velocity through a positive pump (M03-S, Hydra-cell, Minneapolis, MN). The permeate was collected in a container placed on an electronic balance. The reading of the balance was continually recorded at 30 s intervals by a computer. The cross-flow velocity used in the experiments was 0.5 m/s. The transmembrane pressure levels used were 1.4, 2.1, and 2.8 MPa. Two nanofiltration membranes (DS-5DK and DS-5HL, Osmonics) were used in the experiments. Both the membranes could retain 98% of MgSO4 but had different levels of permeate flux. No MWCO information was provided by the manufacturer. The surface area of the membrane is 140 cm^2. The hold up volume of the membrane unit is 70 mL. The nanofiltration process lasted for 1 h.

An alkali–acid treatment method was applied to the membrane system in the following steps: (a) fully open the recirculation and permeate valves, (b) flush with tap water for 5 min, (c) circulate 2 L of 4% phosphoric acid for 10 min, (d) rinse with tap water for 5 min, (e) circulate 2 L of 0.1 N NaOH solution for 10 min, and (f) rinse with 10 L of deionized water for 5 min.

Semicontinuous Fermentation

The fermentation was conducted in a stirred 1.5-L benchtop fermentor. The pH of the broth was maintained at the designated value by neutralizing the acid with 10 *N* ammonium hydroxide during fermentation. The agitation speed of the fermentor was maintained at 200 rpm, whereas the temperature was maintained at 37°C. Samples were withdrawn every 2 h during the first 6 h and every 12 h during the remaining fermentation process.

The preliminary semicontinuous fermentation experiments without membrane separation were conducted at pH 5.0–6.5 for 48 h. After 48 h, half of the broth (0.75 L) was pumped out and same amount (0.75 L) of fresh cheese whey (5%) was added. The fermentation lasted for another 48 h at the same condition. In the semicontinuous fermentation experiments with membrane separation, 0.75 L of broth was harvested from the fermentor using the nanofiltration unit described above. The 5DK membrane was used in the tests. The transmembrane pressure of the tests was maintained at 2.1 MPa. After membrane separation, 0.75 L of fresh cheese whey (5%) was added to replace the removed permeate. The membrane unit was flushed with boiling water for sterilization before each run of separation. In

order to compare the performance of semicontinuous fermentation without and with membrane separation, 0.75 L of broth was also harvested and replaced with 0.75 L of fresh cheese whey every 24 h in the semicontinuous fermentation without membrane separation. The fermentation experiments were replicated three times.

Analysis

Lactose, lactic acid, and acetic acid were measured by high-performance liquid chromatography (Waters, Milford, MA) with a KC-811 ion exclusion column and a Waters 410 differential refractometer detector. The mobile phase was 0.1% H_3PO_4 solution at a flow rate of 1 mL/min. The temperatures of the detector and of the column were maintained at 35°C and 60°C, respectively. The performance of fermentation was evaluated with lactic acid productivity:

$$\text{Lactic acid productivity} = \frac{\text{Lactic acid produced (g / L)}}{\text{Total fermentation time (h)}} \quad (1)$$

The performance of membrane separation was evaluated by using three criteria: (a) permeate flux, (b) lactose retention, and (c) lactic acid recovery. The permeate flux was calculated by measuring the quantity of permeate collected during a certain time and dividing it by the effective membrane area for filtration.

$$\text{Permeate flux } (\text{Lm}^{-2}/\text{h}) \frac{\text{Permeate volume}}{\text{Membrane area} \times \text{time}} \quad (2)$$

The lactose retention (%) was defined as:

$$\text{Lactose retention} = \left(1 - \frac{\text{Concentration of lactose in the permeate}}{\text{Concentration of lactose in the feed stream}}\right) \times 100 \quad (3)$$

The lactic acid recovery (%) was defined as:

$$\text{Lactose recovery} = \frac{\text{Concentration of lactic acid in the permeate}}{\text{Concentration of lactic acid in the feed stream}} \times 100 \quad (4)$$

Results and Discussion

Semicontinuous Fermentation Without Membrane Separation

The preliminary semicontinuous fermentation without membrane separation was conducted at different pH to obtain the optimum fermentation condition. The averaged lactose, lactic acid, and acetic acid concentrations obtained during the fermentation with free cells of *B. longum* at different pH are shown in Figs 1–4. The results show that lactose conversion ratio was significantly affected by the pH ($p < 0.0001$). The lactose

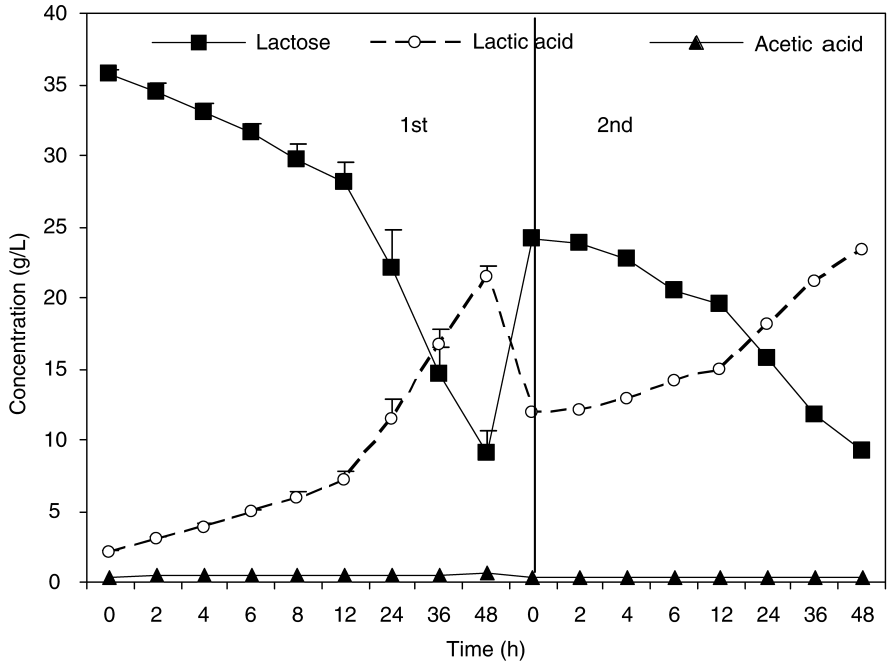

Fig. 1. Semicontinuous production of lactic acid from cheese whey (pH 5.0).

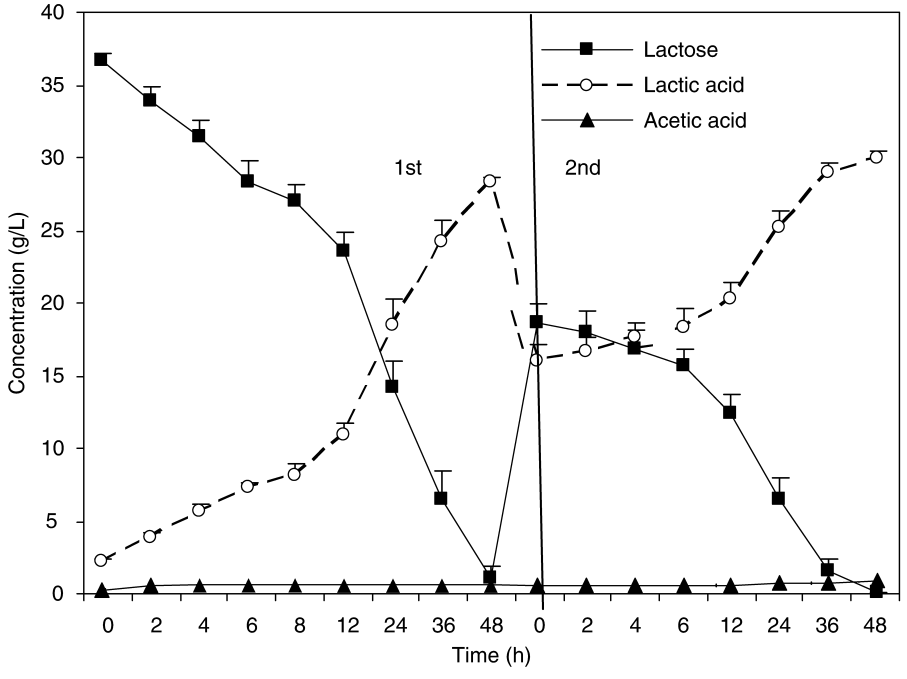

Fig. 2. Semicontinuous production of lactic acid from cheese whey (pH 5.5).

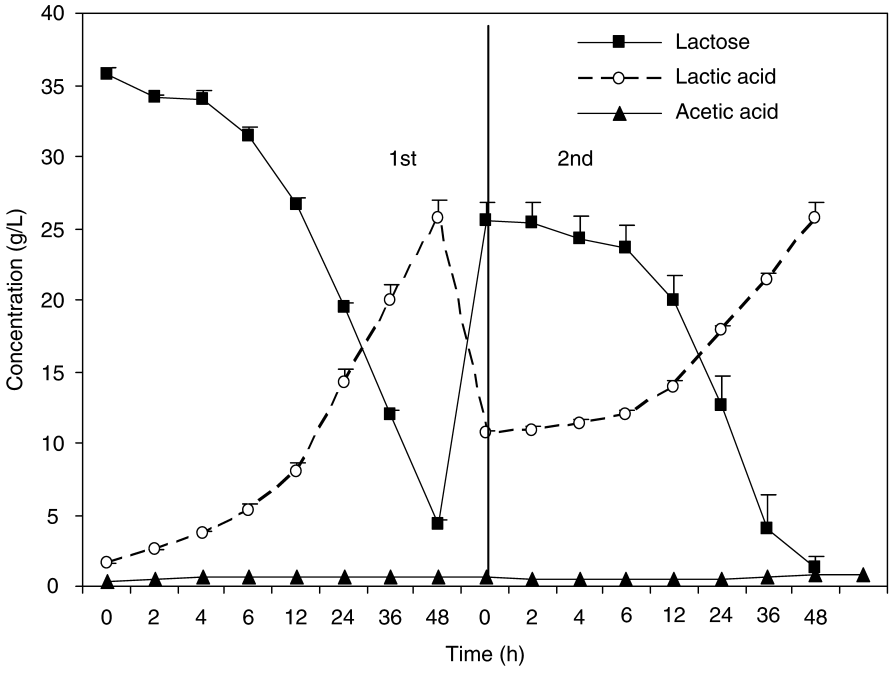

Fig. 3. Semicontinuous production of lactic acid from cheese whey (pH 6.0).

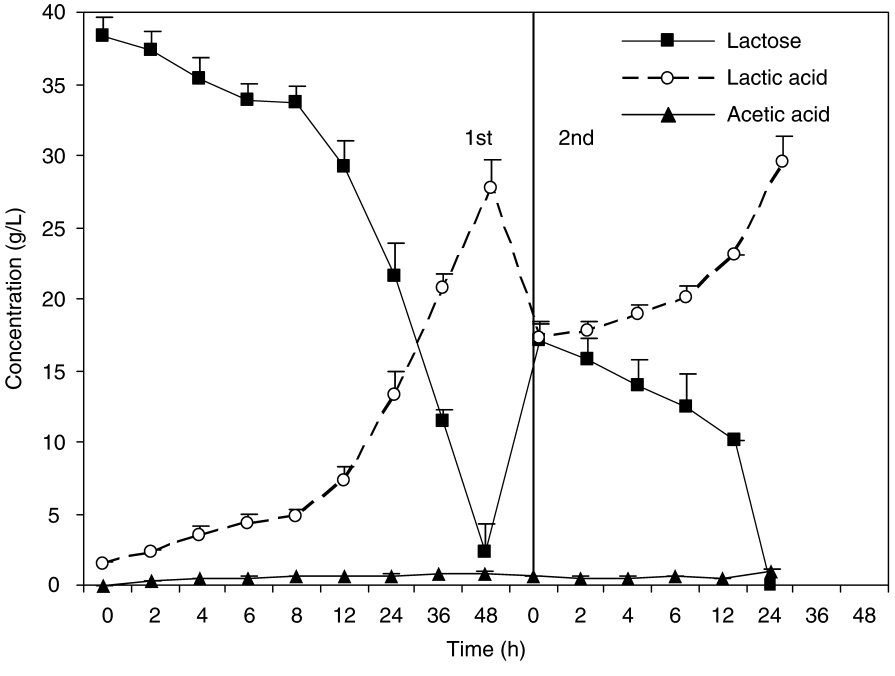

Fig. 4. Semicontinuous production of lactic acid from cheese whey (pH 6.5).

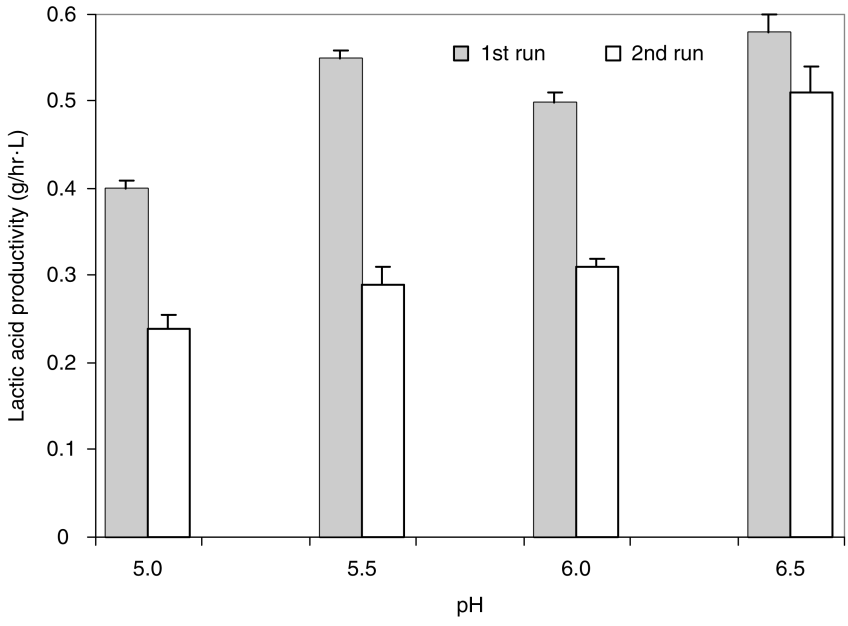

Fig. 5. Lactic acid productivity of the semicontinuous fermentation of cheese whey without membrane separation.

conversion ratio of 75, 97, 88, and 94% was obtained at pH 5.0, 5.5, 6.0, and 6.5 for the first run, respectively. The lactose conversion ratio of 62, 100, 89.5, and 100 was obtained at pH 5.0, 5.5, 6.0, and 6.5 for the second run, respectively. The lactic acid yield was between 0.72 and 0.76 g/g at pH values from 5.0 to 6.5. The pH has no significant effect on the lactic acid yield within the pH range of 5.0–6.5 ($p > 0.2$). The production of acetic acid was negligible in comparison with that of lactic acid production. As the initial lactose concentration of the second run was lower than that of the first run, most of the lactose could be converted during the second run. It can be seen from Fig. 5 that lactic acid productivity of the second run is much lower than that of the first run.

Nanofiltration of Fermentation Broth

The nanofiltration testing results of permeate flux, lactose retention, and lactic acid recovery are shown in Table 1. The values in Table 1 are the average of two replicates. After 48 h of fermentation, nearly all the lactose in the fermentation was converted. In order to evaluate the performance of the membrane on the lactose retention, the lactose concentration in the broth was also adjusted to 10.0 g/L in some tests. It can be seen from Table 1 that when the membrane of 5DK was used, 100% of the lactose was retained at pressure of 2.1 and 2.8 MPa. Under the studied conditions, the membrane of 5HL could only retain about 70% of the lactose. In order to obtain pure lactic acid, membrane of 5DK was selected for the semicontinuous fermentation

Table 1
Permeate Flux, Lactose Retention, and Lactic Acid Recovery During Nanofiltration

Membrane type	Initial concentration (g/L)		Pressure (MPa)	Flux (Lm^{-2}/h)	Lactose retention (%)	Lactic acid recovery (%)
	Lactose	Lactic acid				
5DK	0	25.0	1.4	36.9	–	44.5
	0	25.0	2.1	45.4	–	34.2
	0	25.0	2.8	50.5	–	31.7
	10.0	30.0	1.4	21.9	98.0	44.2
	10.0	30.0	2.1	53.5	100	37.7
	10.0	30.0	2.8	59.3	100	30.0
5HL	0	25.0	1.4	56.9	–	67.8
	0	25.0	2.1	58.1	–	56.0
	0	25.0	2.8	62.6	–	44.2
	10.0	30.0	2.1	50.4	69.0	70.8
	10.0	30.0	2.8	62.0	73.1	62.4

tests. It can be seen from Table 1, that permeate flux and lactose retention increased with the increase of transmembrane pressure. The lactic acid recovery in the permeate decreased with the increase of transmembrane pressure.

Comparison of Semicontinuous Fermentation With and Without Nanofiltration

Figures 6–8 show the comparison of semicontinuous fermentation with and without nanofiltration. As the nanofiltration membrane could retain all the lactose, it can be seen from Fig. 7 that the initial lactose concentrations of the second and third runs of the tests were very close to that of the first run, whereas the lactose concentrations of the second and third runs were lower than that of the first runs in the tests without nanofiltration (Fig. 6). In the tests without nanofiltration, the lactic acid productivity of the second and third run was much lower than that of the first run (Figs. 6 and 8), whereas no decrease in lactic acid productivity was observed in the second and third runs when the nanofiltration membrane was used (Figs. 7 and 8). As the nanofiltration membrane could retain all the cells in the reactor, the initial cell density of the second and third runs with nanofiltration is much higher than that of tests without nanofiltration. It can be seen from Fig. 7 that the lactic acid concentration was increasing with runs, which was caused by the lower lactic acid recovery of the membrane. The selected membrane (5DK) could retain all the lactose, but it also had high lactic acid retention. The membrane need to be modified to increase the lactic acid recovery (reduce the lactic acid retention), whereas retain all the lactose in the future studies.

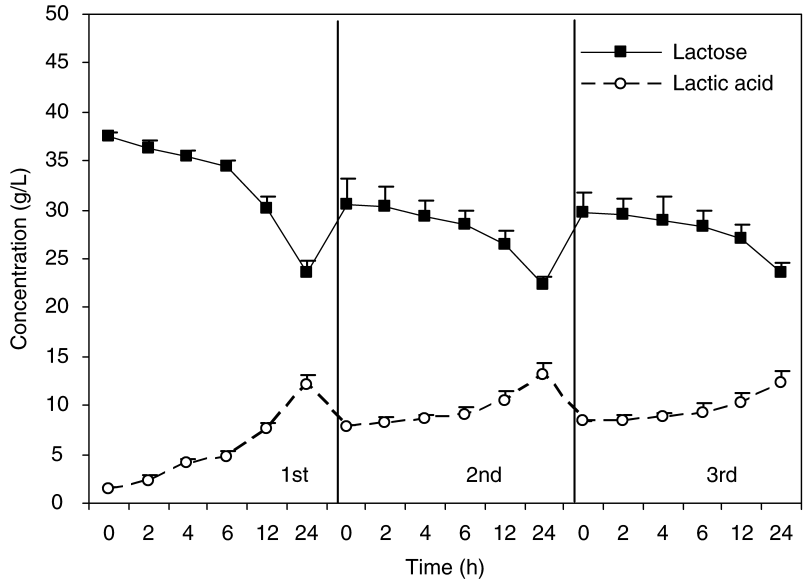

Fig. 6. Semicontinuous lactic acid production without nanofiltration (pH 5.5).

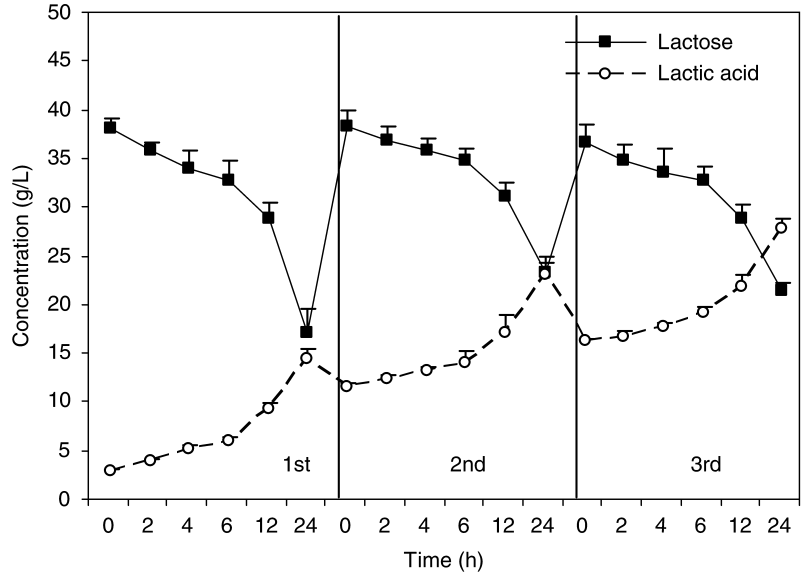

Fig. 7. Semicontinuous production of lactic acid from cheese whey with nanofiltration (pH 5.5).

Conclusion

The semicontinuous fermentation without nanofiltration was tested at pH 5.0–6.5 and high lactose conversion and lactic acid yield were obtained at pH 5.5–6.5 in the first runs. The lactic acid productivity of the second runs was much lower than that of the first runs when the semicontinuous

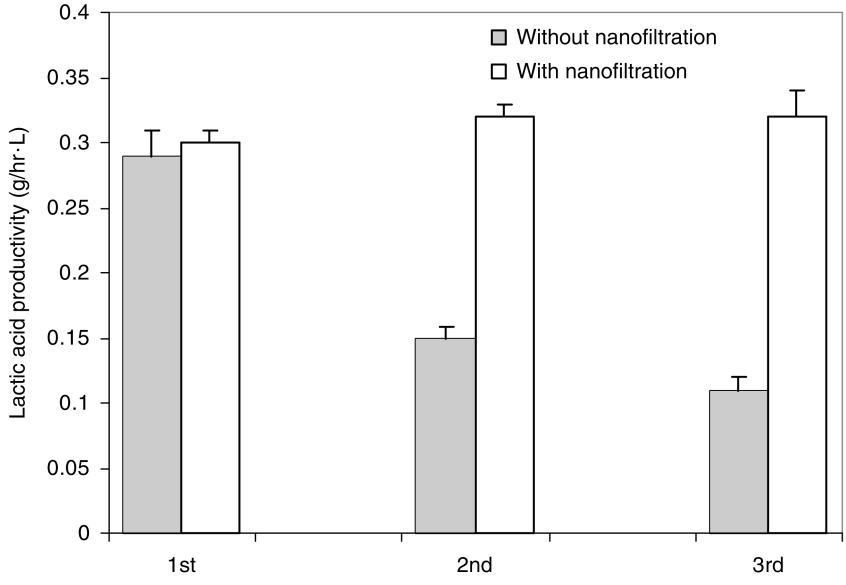

Fig. 8. Comparison of lactic acid productivity of semicontinuous fermentation with and without nanofiltration (pH 5.5).

fermentation was conducted without nanofiltration. Lactose retention ratio of 100% and lactic acid recovery of 38% was obtained with nanofiltration membrane of 5DK at pressure of 2.1 MPa. Membrane of 5HL could only retain about 73% of lactose, but could recover 62% of the lactic acid. When the nanofiltration membrane unit (5DK membrane) was integrated with the fermentor to harvest lactic acid every 24 h, higher lactic acid productivity was obtained for the second and third runs than the first run during the semicontinuous fermentation. Compared with the semicontinuous fermentation without nanofiltration, the lactic acid productivity of the subsequent runs increased.

References

1. Jeantet, R., Maubois, J. L., and Boyaval (1996), *Enzyme Microbial. Technol*. **19,** 614–619.
2. Shahbazi, A., Mims, M. R., Li, Y., Shirley, V., Ibrahim, S. A., and Morris, A. (2005), *Appl. Biochem. Biotechnol.* **121–124,** 529–540.
3. Diosady, L. L. and Puzanov, T. (2005), *Int. J. Appl. Sci. Eng*. **3(1),** 19–25.
4. Bruno-Barcena, J. M., Ragout, A. L., Cordoba, P. R., and Sineriz, F.(1999), *Appl. Microbiol. Biotechnol.* **51,** 316–324.
5. Senthuran, A., Senthuran, V., and Hatti-Kaul, R. (1999), *J. Biotechnol.*, **73,** 61–70**.**
6. Mostafa, N. A. (1995), *Energy Convers. MGMT*. **37(3),** 253–260.
7. Kosikowski, F. V. (1979), *J. Dairy Sci.* **61,** 1149–1160.
8. Siso, M. I. G. (1996), *Bioresour. Technol*. **57,** 1–11.
9. Tango, M. S. A. and Ghaly, A. E. (2002), *Appl. Microbiol. Biotechnol.* **58,** 712–720.
10. Amrouche, T., Boutin, Y., and Moroni, O., (2006), *J. Microbiol. Methods*. **65 (1),** 159–170.
11. Baricault, L., Denariaz, G., Houri, J. J., Bouley, C., Sapin, C., and Trugnan, G. (1995), *Carcinogenisis* **2,** 245–252.

12. Doleyres, Y., Paquin, C., LeRoy, M., and Lacroix, C. (2002), *Appl. Microbiol. Biotechnol*. **60,** 168–173.
13. Song, S. H., Kim, T. B., Oh, H. I., and Oh, D. K. (2003), *World J. Microbiol. Biotechnol.* **19,** 721–731.
14. Van der Horst, H. C. (1995), *Int. Dairy Fed. Spec. Issue* **9504,** 36–52.
15. Eriksson, P. (1988), *Environ. Prog*. **7,** 58–62.
16. Alkhatim, H. S., Alcaina, M. I., Soriana, E. Iborra, M. I., Lora, J., and Arnal, J. (1998), *Desalination* **199,** 177–184.